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Odors eliciting fear: A conditioning approach to Idiopathic Environmental Intolerances

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ABSTRACT

Patients suffering from Idiopathic Environmental Intolerances (IEI) report health symptoms, referable to multiple organ systems, which are triggered by harmless odors and therefore medically unexplainable. In line with previous research that predominantly points towards psychological explanations, the present study tests the hypothesis that IEI symptoms result from learning via classical conditioning of odors to fear. A differential conditioning paradigm was employed. Hedonically different odors were compared on ease of fear acquisition. Conditioned stimuli (CSs) were Dimethyl Sulfide (unpleasant) and peach (pleasant). The unconditioned stimulus (US) was an electrical shock. During acquisition one odor (CS+) was followed by shock, while the other odor (CS−) was not. Next, fear extinction was tested by presenting both CS+ and CS− without US. Electrodermal response, odor evaluation, and sniffing behavior were monitored. Results showed successful fear conditioning irrespective of hedonic character as evidenced by electrodermal response. Acquired fear did not extinguish. There was no evidence of evaluative conditioning taking place, as CS evaluation did not change during fear acquisition. Early avoidance of the CS+, as deduced from odor inhalation measures, was demonstrated, but did not sustain during the entire acquisition phase. This study suggests that a fear conditioning account of IEI is only partially satisfactory.

1. Introduction

Patients suffering from Idiopathic Environmental Intolerances (IEI) — formerly known as Multiple Chemical Sensitivities (MCS, see AAAAI Board of Directors, 1999) — typically respond to a wide range of chemical stimuli, often characterized by a distinctive odor, with medically unexplained symptoms (Leznoff, 1997). Symptoms include allergy-like reactions (eye, nose, and throat irritation; Shusterman, 2003) as well as non-specific complaints, referable to multiple organ systems (e.g. nausea, muscle ache, headache; Labarge & McCaffrey, 2000). Notably, IEI sufferers report symptoms which they attribute to extremely low doses of various chemically unrelated substances in the environment (Bailer, Rist, Witthöft, Paul, & Bayerl, 2004).

IEI is not an accepted health condition in most countries, which complicates estimating its prevalence. Based on a random sample of 19–69 year old individuals in Denmark, Drimer Berg, Linneberg, Dirksen, and Elberling (2008) found that 27% reported symptoms from inhalation of airborne chemicals. In a representative study of the German adult population, 9% agreed with the statement "When I am exposed to chemicals my body reacts immediately" (Hausteiner, Bornschein, Hansen, Zilker, & Förstl, 2005). It may be concluded that these prevalences justify the scientific investigation of chemical sensitivities.

A likely contributor to the poor recognition of IEI is the obscurity of its etiology. Consequently, differential diagnosis by physicians is complicated (Labarge & McCaffrey, 2000), which, in turn, impedes treatment success. While some researchers advocated a biological cause (e.g. that immunological deficits underlie IEI symptoms; Graveling, Pilkington, George, Butler, & Tannahill, 1999; Miller & Mitzel, 1995), others have championed a psychological explanation (Das-Munshi, Rubin, & Wessely, 2006; Labarge & McCaffrey, 2000). According to Leznoff (1997) IEI is best regarded as an anxiety syndrome.

It has been proposed that classical conditioning is involved in the origins of IEI (Shusterman, Balmes, & Cone, 1988; Siegel & Kreutzer, 1997). This model suggests that an odor smelled while experiencing an aversive stimulus (unconditioned stimulus (US); e.g. irritation) that elicits an unconditioned response (UR; e.g. distress) becomes a conditioned stimulus (CS). On a next encounter, the odor (CS) activates an expectation of the US. The individual then

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reacts with a conditioned response (CR: e.g. stress symptoms). As illustration, Shusterman (2001) described a case in which a one-time overexposure to Phosphine-gas (US) led to panic and symptoms consistent with fear-and-stress arousal (UR/CR) in response to subsequent encounters with low concentrations (CS). Arousal symptoms (i.e. insomnia, irritability, and restlessness; Bryant & Harvey, 1998) are common to IEI (Labarge et al., 2000) and also known to result from long lasting periods of sympathetic nervous system (SNS) activation. SNS activation is part of the fear response (Fight-or-flight response, Canon, 1932; in Taylor et al., 2000), and is characterized by elevated heart rate and electrodermal response (EDR). In the present study we investigated EDR as a biomarker of fear (see Section 2.3.1.).

In conclusion, IEI may be conceptualised as some sort of phobia, where the CS is typically olfactory and the feared US is a chemical exposure. The present study aimed to test whether fear conditioning of an odor as CS to an aversive US – an electrical shock – results in specific physiological, cognitive and behavioral responses, that may be expected to play a role in IEI. A differential fear conditioning paradigm was implemented using healthy participants, in which a CS+ odor presentation, not a CS− odor, was followed by electrical shock.

Whereas sufferers from IEI have been known to report health symptoms in response to unpleasant odors, odors considered as pleasant by many such as perfumes may also evoke complaints. Marinkovic, Schell, and Dawson (1989) previously demonstrated classical conditioning of elevated EDR to pleasant odors: presentations of perfumes that had been paired with electrical shock led to elevated EDR at subsequent 'odor-alone' encounters. Van den Bergh and his group, on the other hand, (e.g. Van den Bergh, Kempynck, Van de Woestijne, Baeyens, & Eelen, 1995; Van den Bergh, Stegen, & Van de Woestijne, 1997; Van den Bergh et al., 1999) repeatedly found successful conditioning of (respiratory) symptoms to unpleasant odors, but not to pleasant odors. However, successful conditioning to a pleasant odor could be established when participants were previously warned about environmental pollutions (Winters et al., 2003).

As IEI patients report symptoms to both unpleasant and pleasant odors, the current study investigated differences with respect to the ease at which fear acquisition and extinction develop to hedonically pleasant versus unpleasant stimuli. Thus, as first aim of this study we investigated whether we could replicate the results by Marinkovic et al. (1989), who showed successful fear learning to a pleasant odor, extending it by comparing the results for both pleasant and unpleasant odors over both an acquisition and extinction phase in the same individuals.

There were two further aims to the present study: the second aim, in addition to testing changes in the *expectation* that pleasant odors would be followed by adverse effects, we tested whether there were concomitant changes in *evaluation* of these odors. One of the most striking features of IEI is a dislike of odors, even of odors that are considered pleasant by most people such as from deodorants, detergents, eau de toilette, and perfumes. So, in terms of Pavlovian learning, it seems that *evaluative* conditioning (as in Baeyens, Wrzesniewski, De Houwer, & Eelen, 1996, who showed that individuals who dislike going to the toilet acquired a dislike for the odor experimentally paired with the toilet room) is taking place. To investigate whether a 'hedonic shift' towards unpleasantness occurred during aversive conditioning, pleasantness reports to each of the odors were obtained during the acquisition and extinction phase. We expected that the pleasant odor would be liked less after the classical conditioning phase.

As third aim, we intended to investigate whether classical conditioning of a CS odor to a US leads to avoidance of the odor. Aside from physiological (SNS activation) responses to fearful stressors, avoidance behavior is an objective measure and defining behavioral feature of anxiety (Mowrer, 1947). In order to assess avoidance of the CS, *sniffing* was measured. Sniffing can be considered as active stimulus sampling behavior, in this case of the airborne stimulus. It is regarded as the equivalent of eye movements as an indication of active orientation towards or away from the stimulus (Mainland & Sobel, 2006). Our final and third prediction held that sniffing behavior is changed following classical conditioning, in that total inhalation of CS+ (calculated by frequency, amplitude, duration and latency of sniffs), but not CS−, will decrease in the acquisition phase.

In sum, we aimed to test 1) the extent to which fear can become associated with a pleasant and unpleasant CS odor, respectively, and extinguishes when the CS odor is never again followed by the US, 2) whether evaluative conditioning can be shown, in particular to pleasant odors, and 3) whether the CS tends to be avoided after fear learning has occurred. The main differences with respect to previous studies hold that we tested whether fear conditioning of odor to electrical shock causes both increased expectations of adverse events following an odor as well as changes in hedonic valence of that odor, for both pleasant and unpleasant odor alike, using a traditional fear conditioning approach. Successful treatments of phobia based on classical conditioning theory have been developed. If the results from this research support the model, existing treatments can be applied to IEI as well, and novel hypotheses based on the model and the present findings can be better generated.

2. Methods[1]

2.1. Participants

Participants were 53 healthy students recruited from Utrecht University (38 women) of at least 18 years old (range 18–30 years; $M = 21.9$, SD = 2.58). Prior to the experiment participants received an information letter. Students were screened for psychiatric conditions, heart conditions, epilepsy, pregnancy, self-reported sense of smell, allergies, and asthma. Also, the modified Chemical Odor Intolerance Index was administered (Bell, Schwartz, Peterson, & Amend, 1993, for the modified version see Dalton, 1999). No participants had scores indicative of IEI (scores of 4 or higher on 3 or more items ranging 1–5). After screening participants were randomly divided over two conditions. Twenty-seven participants (17 women) received peach as the CS+ and 26 (21 women) received Dimethyl Sulfide (DMS) as the CS+.

2.2. Stimuli

The pleasant (peach: Jacob Hooy) and unpleasant (DMS: Quest) odors were used as CSs. Odorant and odor concentrations were selected in a pilot study to be significantly different in hedonic value yet iso-intense. The pilot study ($N = 36$) showed that the odor of peach at a 100% concentration (10 ml) and .125% v/v DMS (10 ml with dipropylene glycol as diluent) were rated as equally intense (paired samples *t*-test, $t(19) < 1$, *n.s.*). Moreover, on a 9-point Likert scale (0 = extremely unpleasant, 9 = extremely pleasant) DMS was rated as relatively unpleasant ($M = 3.85$, SD = 1.58) and the odor of peach was rated as relatively pleasant ($M = 6.27$, SD = 1.14), $t(19) = 6.04$, $p < .001$, $r = .81$.

Odor dilutions (10 ml each) were stored in 250 ml bottles (Schott Duran) and presented through a cup that was located 2 cm under the participant's nose. Low adhesion tubes connected the

[1] The current study's design was approved by the Medical Ethics Committee of University Medical Centre Utrecht (assigned reference: 08/379).

bottles with the cup opening only when bottle valves were opened. A pump-generated airflow over the headspace of the bottles pushed the odorant vapor via tubing towards the cup opening with a speed of 5 l/min (see Fig. 1).

Each odor presentation had a duration of 6 s. Onset of odor presentations was visually announced via a 3 s white cross on a black background on the computer screen at a distance of approximately 40 cm. Depending on the condition CS+ was either the pleasant or unpleasant odor, followed by a US (shock) in the acquisition phase. Odors were refreshed every five participants to ensure stimulus quality.

2.3. Materials

2.3.1. Physiological endpoints

Electrodermal response (EDR) served as the primary physiological endpoint of fear-induced arousal. EDR was registered with a Coulbourn Modular Instruments System (Allentown, PA, USA) via a Coulbourn Isolated Skin Conductance coupler (S71-23). A constant .5 V current through 9-mm Sensor Medics Ag/AgCl electrodes was used. These electrodes were placed on the middle and ring finger of the non-dominant hand in accordance with published guidelines (Fowles et al., 1981). Lead was facilitated by paste that exactly fitted the electrodes.

The aversive US was elicited by administration of electric shocks (duration: 500 ms). Intensity of shock was determined as explained under Section 2.4. Shock electrodes were placed on the middle and ring finger of the dominant hand. The electrodes were separated by 14 mm as determined by the width of the adhesive collar.

To assess sniffing, participants wore a nasal pressure monitoring cannula that is normally used to deliver oxygen to patients in hospitals or nursing homes (Johnson, Russell, Khan, & Sobel, 2006). The cannula was connected to a pressure transducer (PT: Sleep Sense), which registered sniffing behavior by measuring air pressure in the nose (in the range of 0–40 cm H_2O). The PT amplified the signal, which in turn was digitized using a 16 bit analog–digital converter (National Instruments) and processed by the Coulbourn Modular Instruments System.

2.3.2. Psychological measures

Evaluations of odor intensity and pleasantness were assessed via a computer screen directly after each odor presentation by means of a VAS on intensity (range 0–100; 0 = weak, 100 = unbearably strong) and a 9-point Likert scale on pleasantness (range 0–9; 0 = extremely unpleasant, 5 = neither pleasant nor unpleasant, 9 = extremely pleasant).

To investigate the relation between awareness and conditioning success, contingency awareness (i.e. awareness of the CS–US link) was assessed with a forced choice question at the end of the experiment (i.e., "Throughout the experiment, during the presence of which odor did you most expect the electrical shock?"). Participants were presented both odors once, in a counterbalanced order, and confirmed their choice by a mouse click on either "odor 1" or "odor 2" labels that were displayed on the computer screen.

2.4. Procedure

The experiment consisted of an acclimatization phase, a habituation phase, an acquisition phase, an extinction phase, and a post-experimental phase. EDR, sniffing behavior, and odor evaluations were assessed in the habituation, acquisition and extinction phase. During the acclimatization phase informed consent was signed, and the general health and demographics questionnaire were completed. Participants were instructed how to sniff properly: it was emphasized they should breathe and inhale through the nose in a natural manner. The electrodes and the sniffing cannula were applied (by placing the tubing over the head and behind the ears; the cannulas were inserted into each nostril for less than 1 cm) during this phase.

In order to rule out confounding variation in pain sensitivity among participants, a "work-up" procedure was applied in which participants had to select a shock level that was clearly unpleasant, though not painful (cf. Effting & Kindt, 2007). A series of different current flows (starting low, ending higher) was delivered manually by the investigator. The investigator continually increased the shock by a single increment – starting at .2 mA, maxing at 4.0 mA – until the criterion level was reached (based on participant's

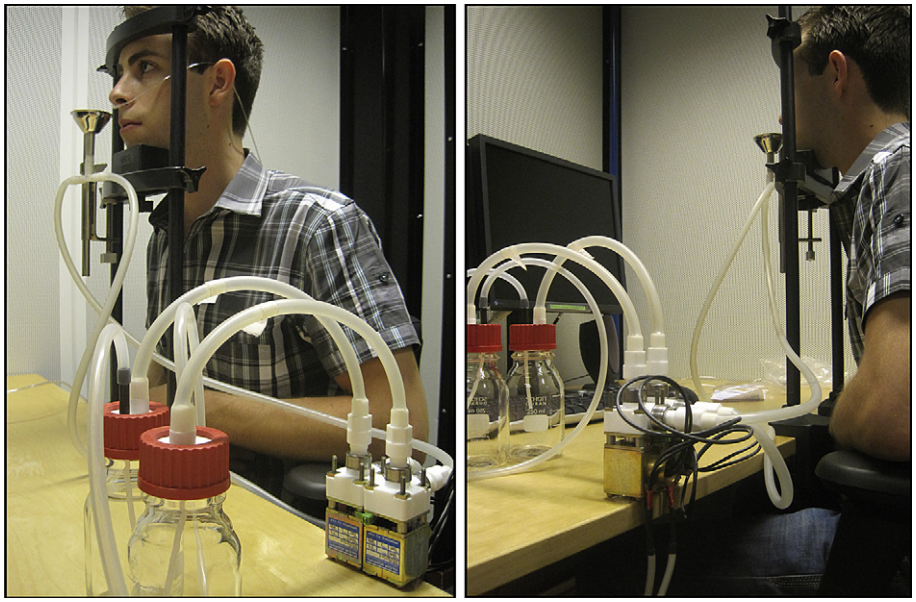

Fig. 1. Photos displaying experimental setup. Headspace of odorant solution is delivered as odor to the participant via depicted cup. Switching of odorants is regulated via valves. Odorant bottles are hidden from view during testing. Sniffing is measured via nasal cannula throughout testing.

feedback). Stimulus level could be increased to .4, .6, .8, 1.1, 1.4, 1.7, 2.0, 2.3, and 4.0 mA.

During the habituation phase both odors were presented 4 times (cf. Milad, Orr, Pitman, & Rauch, 2005). Participants placed their chin in a chin rest 2 cm removed from the odor cup. During the habituation phase baseline physiological reactivity to the CSs was assessed.

During the acquisition phase participants received an electric shock directly following CS+ for 500 ms, but not after the CS−. Both CS+ and CS− were presented 6 times (cf. Milad et al., 2005) in a semi-random manner with a maximum of two similar odor presentations in succession. Depending on the condition, the pleasant odor acted as CS+ and the unpleasant odor as CS− or vice versa.

The extinction phase was similar to the acquisition phase, but without US presentation. The inter-trial interval (ITI) in each phase lasted 60 s and was included to allow physiological recovery from adaptation to the odorant by the odor receptors in the nose. During each ITI, participants first filled out the VASs followed by a simple filler task − tracking a moving circle on the screen − to maintain alertness.

In a post-experimental phase, both odors (CS+ and CS−) were presented for 6 s in a counterbalanced order. In order to assess contingency awareness, participants indicated after which of the two odors they expected the shock most. After the experiment, participants were debriefed, thanked for their participation, and they received financial compensation.

2.5. Data preparation and data analysis

Change in EDR was calculated by subtracting the EDR level at 5 s before CS-onset from the highest EDR peak in a latency window of 6 s directly following CS-onset. Milad et al. (2005) used the mean EDR value at 2 s prior to CS-onset as baseline. However, since the 3 s visual announcement of each CS in the present experiment may have influenced the EDR, we decided to use the EDR value 2 s prior to the visual announcement as baseline. A minimum response criterion of .5 μS was used (Effting & Kindt, 2007). EDR scores were corrected for individual differences in response to US by using the following formula: ([CS_{score}] − [Pre-CS_{score}])/([highest US_{score}] − [Pre-CS_{score} preceding highest US]). Since the EDR scores showed positively skewed distributions, all EDR scores were transformed by calculating square rooted scores.

Sniffing endpoints included sniff frequency, amplitude, duration and latency during a time window of 6 s directly following CS-onset. Area under the curve (AUC) based on these measures was further analyzed, based on Johnson et al. (2006) that this was the most sensitive sniff measurement technique (compared to maximum value and mean value). Data were analyzed by a computer program (Sniff Pressure Analyzer, version 2.7; developed by our lab technician) that is capable of automatically scanning data in search for sniff pulses. Additionally, all sniffing data were visually inspected by the investigator. Thus, sniffs that were not automatically detected due to software limitations could be included in the analyses. AUC scores showed negatively skewed distributions. Therefore, all AUC scores were log transformed by using the following formula: log 10(AUC_{score} + 1). The constant value of '1' was added to allow log values of zeros (Field, 2005).

All analyses were performed using SPSS 16.0. Repeated measures ANOVAs were used for each phase separately to investigate both pre-existing differences and change in EDR, sniffing behavior, and odor evaluation. Data were analyzed with Condition (pleasant vs. unpleasant odor as CS+) as a between-group factor and CS Type (CS+ vs. CS−) as well as Trial (i.e. the various measurement points) as within-group factors. For ANOVAs on the acquisition and extinction phase only the first and last trial of the corresponding phase were included as the within-group factor Trial (Effting & Kindt, 2007; Hermans, Vansteenwegen, Crombez, Baeyens, & Eelen, 2002). For further specification of present interaction effects post-hoc paired samples *t*-tests were performed, applying Bonferroni corrections. In all ANOVAs Greenhouse-Geisser corrections ($\varepsilon < .75$) or Huynh−Feldt corrections ($\varepsilon > .75$) were used in case the assumption of sphericity was violated, as indicated by Mauchly's test (Field, 2005).

Unfortunately, during preparation of the odor dilutions an error was made. DMS was prepared in a 1.25% v/v concentration instead of a .125% v/v concentration. Odor intensity can interfere with odor pleasantness as some odors are experienced as less pleasant solely because they are more intense or vice versa (Bensafi et al., 2002). Consequently, confounds in interpreting the relation between hedonic quality of the odor and successfulness of fear conditioning might occur. To avoid such confounds, repeated measures ANOVAs on EDR and sniffing data comparing the two conditions using a covariate ($\Delta_{INTENSITY}$) were conducted.

$\Delta_{INTENSITY}$ refers to the a-priori difference in intensity between peach and DMS based on the average intensity ratings over the 4 habituation trials. In this manner we were able to differentiate between effects in EDR and sniffing between conditions based on classical conditioning and hedonic value of the CS+ versus on effects in these parameters based on intensity. Results from covariation analyses did not lead to different conclusions than did results from analysis not including the covariate. The statistics reported below for EDR and sniffing include the covariate $\Delta_{INTENSITY}$.

3. Results

3.1. EDR as a result of classical conditioning

In line with the first aim of the study, which was to establish whether odors can elicit fear by classical conditioning to electric shock, EDR results are first reported. Selected shock level was not different across conditions, $t(51)<1$, *n.s.* Mean shock level of the US as selected by participants was 1.7 mA (range: .6−4.0 mA). All participants had contingency awareness, as they all correctly indicated which odor was followed by shock after the experiment.

Pre-existing differences in EDR between the odorants peach and DMS were checked: A 4 (Trial: H1, H2, H3, H4) × 2 (Odorant: Peach vs. DMS) ANOVA on EDR showed differences in EDR levels between the odors during habituation, $F(1,51) = 7.04$, $p < .05$, $\eta_p^2 = .12$. Post-hoc paired samples *t*-tests indicated that EDR levels were significantly higher for DMS than Peach at all habituation trials. These findings correspond with studies reporting higher baseline EDRs to unpleasant odors as opposed to pleasant odors (Alaoui-Ismaïli, Robin, Rada, Dittmar, & Vernet-Maury, 1997; Alaoui-Ismaïli, Vernet-Maury, Dittmar, Delhomme, & Chanel, 1997).

Fig. 2A illustrates EDR levels during CS− and CS+ presentations throughout the experiment. Table 1 shows original EDR recordings (before transformations) during CS+ and CS− presentations from the habituation, acquisition, and extinction phase, pooled over conditions.

3.1.1. Habituation

Table 2 gives an overview of test results for EDR. During habituation EDRs to CS+ and CS− were still comparable, as might be assumed: There was no main effect for CS Type, $F < 1$. A significant main effect of Trial was found reflecting decreasing arousal as participants became acquainted with the odors and the procedure (see Fig. 2). There was no Trial × CS Type interaction effect.

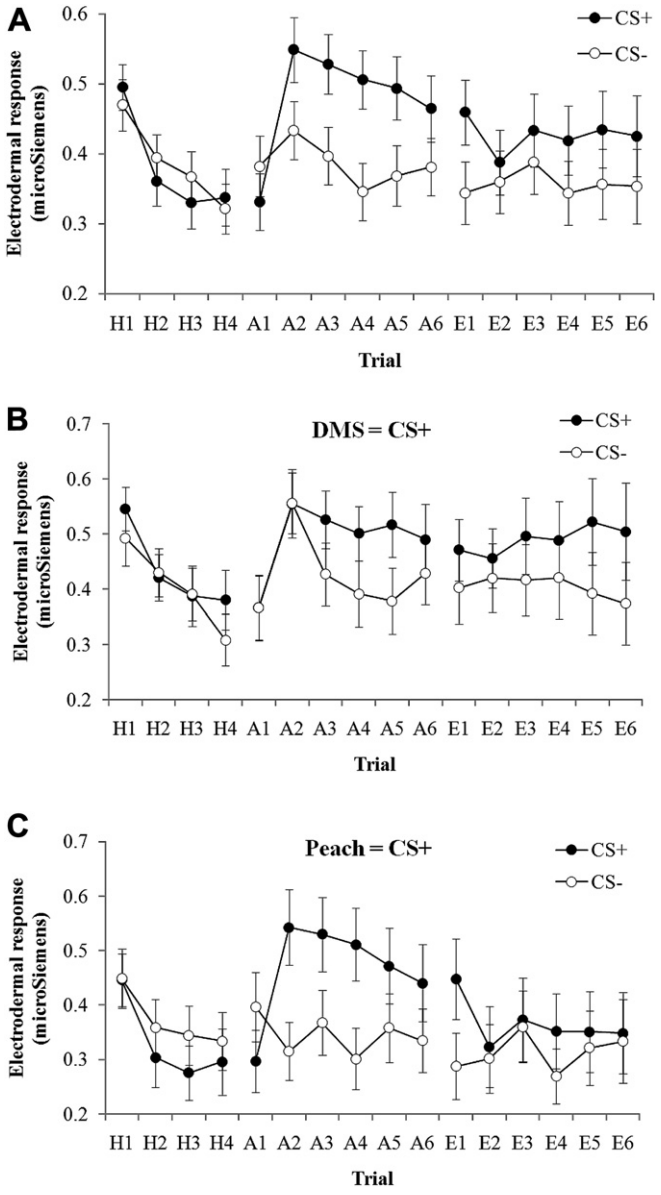

Fig. 2. Change in electrodermal response levels for CS+ and CS− during habituation (H1–H4), acquisition (A1–A6), and extinction (E1–E6). Responses are square rooted means corrected for individual US-responses and baseline-responses. Data are shown collapsed across odors (A), and separately for both conditions, with DMS (B) or peach (C) serving as CS+.

Table 1
Original electrodermal response recordings in MicroSiemens during the habituation phase, acquisition phase, and extinction phase.[a]

Trial	1	2	3	4	5	6
Habituation						
CS−	.57 (.89)	.42 (.43)	.40 (.54)	.32 (.37)		
CS+	.61 (.61)	.37 (.40)	.37 (.43)	.38 (.54)		
Acquisition						
CS−	.53 (.80)	.53 (64)	.51 (.61)	.40 (.53)	.48 (.69)	.42 (.52)
CS+	.37 (.55)	.75 (.90)	.73 (.93)	.71 (.80)	.70 (.93)	.67 (.87)
Extinction						
CS−	.41 (.59)	.46 (.63)	.51 (.74)	.43 (.73)	.48 (.68)	.49 (74)
CS+	.61 (.73)	.54 (.76)	.61 (.86)	.60 (.80)	.65 (.87)	.67 (.93)

Note. Table represents data pooled over conditions.
[a] Values are means (SD).

Table 2
Results of repeated measures ANOVA on electrodermal responses with $\Delta_{INTENSITY}$ as covariate.

Factors	df	Error df	F	η_p^2
Habituation phase				
Trial (H1, H2, H3, H4)	2.32	118.18	14.95***	.23
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	3	153	<1	
Acquisition phase				
Trial (A1 vs. A6)	1	50	7.67**	.13
CS Type (CS+ vs. CS−)	1	50	<1	
Trial × CS Type	1	50	10.50**	.17
Trial × Condition	1	50	1.03	
CS Type × Condition	1	50	<1	
Trial × CS Type × Condition	1	50	2.43	
Extinction phase				
Trial (E1 vs. E6)	1	50	<1	
CS Type (CS+ vs. CS−)	1	50	12.39**	.20
Trial × CS Type	1	50	1.02	
Trial × Condition	1	50	<1	
CS Type × Condition	1	50	<1	
Trial × CS Type × Condition	1	50	5.93*	.11
Condition: Peach = CS+				
Trial (E1 vs. E6)	1	25	<1	
CS Type (CS+ vs. CS−)	1	25	5.42*	.18
Trial × CS Type	1	25	4.81*	.16
Condition: DMS = CS+				
Trial (E1 vs. E6)	1	24	1.74	
CS Type (CS+ vs. CS−)	1	24	11.15**	.32
Trial × CS Type	1	24	<1	

Note. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$.

3.1.2. Acquisition

The covariate $\Delta_{INTENSITY}$ was not significant, $F < 1$. Decomposition of the Trial × CS Type interaction revealed that fear acquisition was successful. At A1 participants were equally aroused during CS+ and CS− presentations ($t(52) = 1.54$, *n.s.*); at A6 participants were more aroused during CS+ than during CS− ($t(52) = 3.14$, $p < .01$). Only EDR to CS+ increased over time (A1 vs. A6: $t(52) = 3.88$, $p < .0001$), not to CS− ($t(52) < 1$). Crucially, the increase in arousal to CS+ over time was larger than the increase in arousal to CS−, as revealed by a comparison of difference scores (A6 − A1) for both CSs (ΔCS+ vs. ΔCS−: $t(52) = 3.08$, $p < .01$). As the Trial × CS Type × Condition interaction was not significant, no post-hoc tests were performed for the two conditions separately (i.e. Peach as CS+ vs. DMS as CS+). Thus, fear acquisition was successful, irrespective of odor valence.

3.1.3. Extinction

For all ANOVAs on the extinction phase the covariate $\Delta_{INTENSITY}$ was not significant, $F < 1$. The main effect for CS Type reflected overall higher EDR to CS+ than to CS−. Visual examination of Fig. 2B and C, showing EDR changes throughout the experiment for each condition separately, suggests differences in fear extinction between the two conditions. This was confirmed by the significant Trial × CS Type × Condition interaction. Two separate ANOVAs for each condition demonstrated that only for the condition with Peach as CS+ a Trial × CS Type interaction was found (see Table 2).

In the condition with Peach as CS+, at E1 participants were still more aroused during CS+ than during CS− ($t(26) = 3.86$, $p < .001$), but at E6 this difference had disappeared ($t(26) < 1$). EDR to CS+ did not decrease significantly over time (E1 vs. E6: $t(26) = 1.79$, $p = .086$), EDR to CS− was unchanged ($t(26) < 1$). However, the decrease in arousal over time (after Bonferroni corrections) was not larger for CS+ than for CS−, as shown by a comparison of difference scores (E6 minus E1) between the two CSs (ΔCS+ vs. ΔCS−: *t*

(52) = 2.32, $p = .029$). So, even though a decline in EDR to CS+ is suggested in Fig. 2C, we did not find statistical evidence for extinction. In sum, it appears that fear was acquired for both the pleasant and unpleasant odor, but acquired fear did not extinguish within 6 trials, irrespective of odor valence.

3.2. Evaluative conditioning: changes in pleasantness

In accordance with the second aim of the study, it was determined whether evaluative conditioning to the CS+ occurred. Table 3 gives an overview of test results.

3.2.1. Habituation

No differences in odor pleasantness ratings were present between CS+ and CS− during habituation. Differences in pleasantness ratings between CS+ and CS− in the acquisition and extinction phase could therefore be attributed to experimental manipulations. A-priori differences in pleasantness ratings between the odors were present, and were intentional: A 2(Odor: DMS vs. Peach) × 2(Trial: H1, H2, H3, H4) ANOVA on pleasantness ratings showed main effect of Odor, $F(1,52) = 187.06$, $p < .001$, $\eta_p^2 = .78$.

3.2.2. Acquisition

There was a Trial × CS Type interaction (see Table 3; Fig. 3A). Further examination revealed that pleasantness ratings were similar for both CSs at A1 as well as at A6 (for both time points: $t(52) < 1$). Over time, pleasantness ratings did not change for CS+ (A1 vs. A6: $t(52) = 1.63$, $p = .11$), neither for CS− (A1 vs. A6: $t(52) = 1.16$, *n.s.*). Crucially, the decrease of pleasantness ratings was not significantly larger for CS+ than for CS−, as revealed by a comparison of difference scores (A6 minus A1) between the two CSs (ΔCS+ vs. ΔCS−: $t(52) = 2.00$, $p = .05$). Thus, no evidence for evaluative conditioning was found. The CS Type × Condition interaction reflected that regardless of odor type (CS+ vs. CS−) the peach odorant was rated as more pleasant than the DMS odorant, as would be expected.

3.2.3. Extinction

Although, technically, extinction cannot occur in the absence of successful conditioning, analyses were conducted to examine the pattern of evaluations over time. A remarkable shift in CS+ pleasantness ratings can be observed between the last acquisition trial (A6) and the first extinction trial (E1, Fig. 3A). A Trial (A6 vs. E1) × CS Type × Condition ANOVA showed a Trial × CS Type interaction, $F(1,51) = 7.04$, $p < .05$, $\eta^2 = .01$, but no three-way interaction ($F < 1$). Crucially, the shift in CS+ pleasantness ratings was significant ($t(52) = 3.25$, $p < .01$), whereas CS− ratings remained unchanged ($t(52)<1$). A possible explanation for this sudden shift in CS+ ratings is that participants were instantly relieved at trial E1 when they experienced for the first time that CS+ was not followed by shock.

Because of this sudden shift in pleasant ratings between trials A6 and E1 we chose to investigate changes in pleasantness ratings corresponding to the extinction phase by comparing data from the last acquisition trial, A6, with trial E6 (see Table 3). During extinction, the change of pleasantness ratings over time was not different for the two CSs: no Trial × CS Type interaction was found. Only a CS Type × Condition interaction was found with pleasantness ratings for Peach being higher than for DMS in

Table 3
Results of repeated measures ANOVA on pleasantness ratings.

Factors	df	Error df	*F*	η_p^2
Habituation phase				
Trial (H1, H2, H3, H4)	2.62	135.96	2.26	
CS Type (CS+ vs. CS−)	1	52	<1	
Trial × CS Type	2.57	133.76	<1	
Acquisition phase				
Trial (A1 vs. A6)	1	51	<1	
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	1	51	3.92*	.07
Trial × Condition	1	51	<1	
CS Type × Condition	1	51	109.38***	.68
Trial × CS Type × Condition	1	51	<1	
Across phase				
Trial (A6 vs. E6)	1	51	<1	
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	1	51	1.33	
Trial × Condition	1	51	<1	
CS Type × Condition	1	51	94.38***	.65
Trial × CS type × Condition	1	51	<1	

Note. *$p = .03$ (one-tailed), ***$p < .001$.

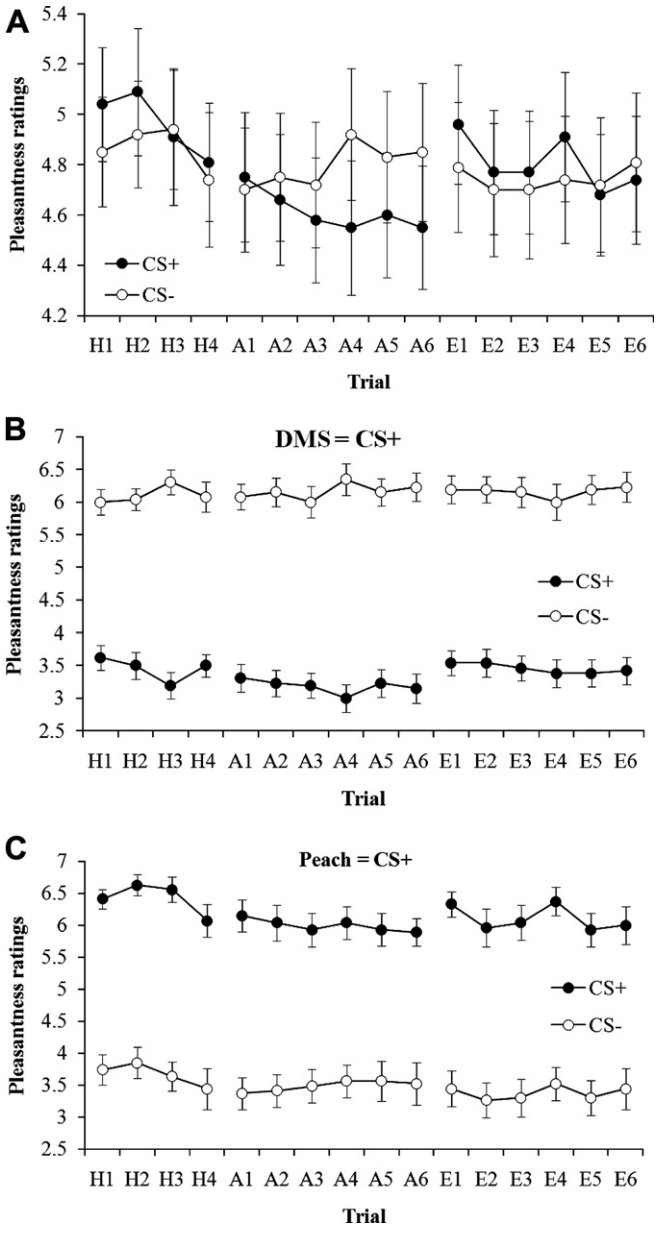

Fig. 3. Change in pleasantness ratings during habituation (H1–H4), acquisition (A1–A6), and extinction (E1–E6), averaged for both conditions. Data are shown collapsed across odors (A), and separately for both conditions, with DMS (B) or peach (C) serving as CS+.

both conditions. No other effects were found. Moreover, CS+ was rated equally (un)pleasant at the beginning of the acquisition phase as compared to the end of the extinction phase (A1 vs. E6: $t(52) < 1$). Together, these findings indicate a lack of evaluative conditioning.

In sum, analyses showed that ratings of odor valence neither changed during fear acquisition, nor during the extinction phase.

3.3. Intensity ratings

3.3.1. Habituation

The course of intensity ratings is visualized in Fig. 4. Table 4 gives an overview of test results. No differences in intensity ratings were present between CS+ and CS− during habituation, prior to acquisition. There was a Trial × CS Type interaction that was manifested by CS− ratings slightly increasing over time and CS+ ratings slightly decreasing. No main effect of trial was found. Perceived intensity differences between the odors were present: A 2(Odor: DMS vs. Peach) × 2(Trial: H1, H2, H3, H4) ANOVA on intensity ratings showed main effect of Odor, $F(1,52) = 25.24$, $p < .001$, $\eta_p^2 = .33$.

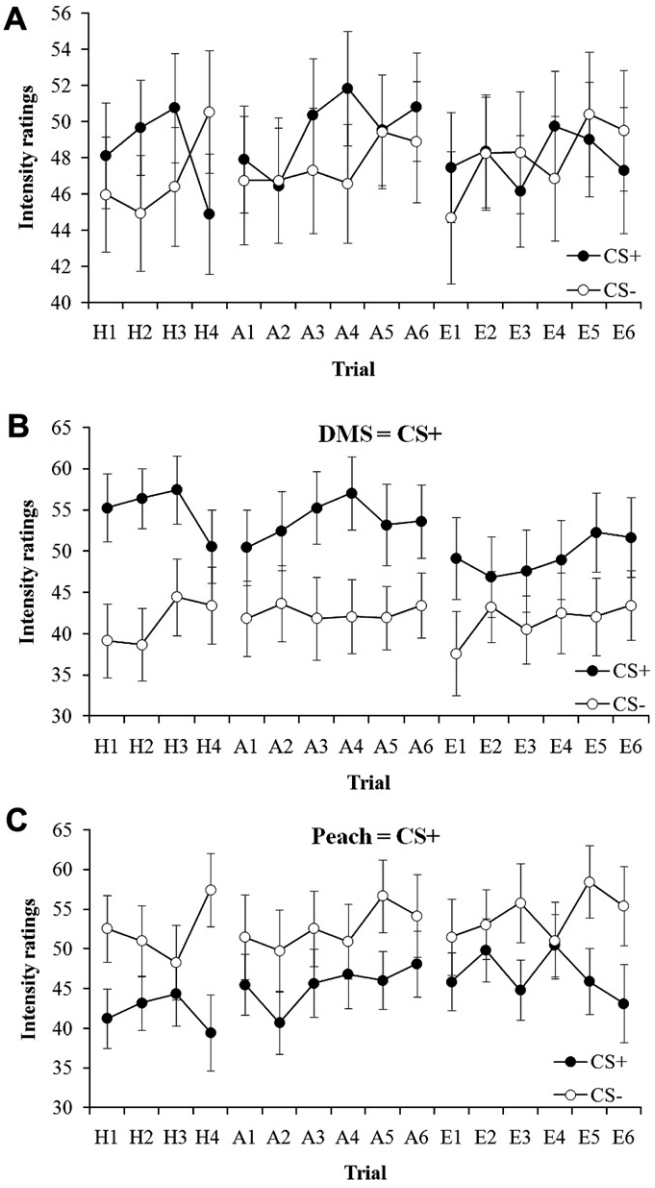

Fig. 4. Change in intensity ratings during habituation (H1–H4), acquisition (A1–A6), and extinction (E1–E6), averaged for both conditions. Data are shown collapsed across odors (A), and separately for both conditions, with DMS (B) or peach (C) serving as CS+.

Table 4
Results of repeated measures ANOVA on intensity ratings.

Factors	df	Error df	F	η_p^2
Habituation phase				
Trial (H1, H2, H3, H4)	3	156	<1	
CS Type (CS+ vs. CS−)	1	52	<1	
Trial × CS Type	3	156	5.04**	.09
Acquisition phase				
Trial (A1 vs. A6)	1	51	2.24	
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	1	51	<1	
Trial × Condition	1	51	<1	
CS Type × Condition	1	51	6.70*	.12
Trial × CS Type × Condition	1	51	<1	
Extinction phase				
Trial (E1 vs. E6)	1	51	1.17	
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	1	51	2.52	
Trial × Condition	1	51	<1	
CS Type × Condition	1	51	14.86***	.23
Trial × CS Type × Condition	1	51	<1	

Note. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$.

3.3.2. Acquisition

DMS was perceived as more intense throughout as shown by a CS Type × Condition interaction. Intensity ratings were higher for DMS when acting as CS+ as well as when acting as CS−. No other effects were found (see Table 4).

3.3.3. Extinction

Similar to the acquisition phase, only a CS Type × Condition interaction effect was found that was manifested by higher intensity ratings for DMS in both conditions. No other effects were found.

In sum, these results indicate that odor intensity ratings did not change during fear acquisition and fear extinction.

3.4. CS avoidance: sniffing behavior

Finally, it was determined if conditioning of odor to fear was accompanied by odor avoidance, using sniffing as a parameter. Table 5 gives an overview of test results.

3.4.1. Habituation

There were no differences in inhalation of the CS+ and CS− odorants prior to fear acquisition as revealed by a nonsignificant main effect of CS Type.

3.4.2. Acquisition

The covariate $\Delta_{INTENSITY}$ was significant, $F(1,50) = 4.12$, $p < .05$. However, analyses run without the covariate $\Delta_{INTENSITY}$ did not differ markedly from those reported in the Table, with the interaction between CS Type and Condition being the only significant effect. Avoidance of CS+ seemed to be present over the first four trials of the acquisition phase (see Fig. 5). In fact, inhalation of CS+ initially decreased (A1 vs. A4: $t(52) = 2.97$, $p < .01$) and was significantly different from inhalation of CS− at A3 ($t(52) = 2.55$, $p < .05$) and A4 ($t(52) = 2.31$, $p < .05$). However, this apparent avoidance effect extinguished over the last two acquisition trials

Table 5
Results of repeated measures ANOVA on sniffing ratings with $\Delta_{INTENSITY}$ as covariate.

Factors	df	Error *df*	*F*	η_p^2
Habituation phase				
Trial (H1, H2, H3, H4)	2.29	116.56	1.20	
CS Type (CS+ vs. CS−)	1	51	<1	
Trial × CS Type	3	153	<1	
Acquisition phase				
Trial (A1 vs. A6)	1	50	<1	
CS Type (CS+ vs. CS−)	1	50	<1	
Trial × CS Type	1	50	1.28	
Trial × Condition	1	50	2.07	
CS Type × Condition	1	50	15.48***	.24
Trial × CS Type × Condition	1	50	2.78	
Extinction phase				
Trial (E1 vs. E6)	1	50	1.53	
CS Type (CS+ vs. CS−)	1	50	<1	
Trial × CS Type	1	50	4.19*	.08
Trial × Condition	1	50	1.15	
CS Type × Condition	1	50	27.05***	.35
Trial × CS Type × Condition	1	50	<1	

Note. $^*p < .05$, $^{***}p < .001$.

(CS+ vs. CS−: $F < 1$). The significant CS Type × Condition interaction indicated that the DMS odorant was inhaled less than the Peach odorant, irrespective of acting as CS+ or CS−. Thus, after correcting for pre-existing sniffing differences between the odors, no additional effect of fear learning was found.

3.4.3. Extinction

The peach odorant was inhaled more than the DMS odorant during the extinction phase, regardless of CS status, as shown by the CS Type × Condition interaction. There was also a CS Type × Trial interaction with sniffing of CS+ not changing over time ($F < 1$) and sniffing of CS− tending to increase over time (E1 vs. E6: $t(52) = 1.76$, $p = .09$). However, this minimal increase can solely be explained by the initial dip of CS− sniffing at E1. No other effects were found.

In sum, sniffing of CS+ neither changed during fear acquisition, nor during the extinction phase.

4. Discussion

In the present study a fear conditioning model using an electrical shock as US and EDR as a measure of autonomic fear response was employed to model mechanisms involved in fear development in IEI. Irrespective of hedonic character of the odor, EDR to CS+ but not to CS− increased as a result of conditioning. Thus, we were successful in replicating the findings by Marinkovic et al. (1989) that pleasant odors can be conditioned to electrical shock yielding elevated EDR levels. Marinkovic et al. emphasized the importance of demonstrating that similar conditioning is also shown in biologically prepared odors; our results demonstrate just this. In addition, this finding supports Shusterman's (2001) conditioning model and fits the literature pointing towards psychological explanations of IEI (Das-Munshi et al., 2006). However, the results that both pleasant and unpleasant odors can be conditioned to fear are not in accordance with findings by Van den Bergh et al. (1995, 1997, 1999) who only found conditioned respiratory effects to unpleasant odors. The most obvious difference, which may underlie this discrepancy, may be between our procedure and theirs. Van den Bergh et al. used CO_2-enriched air as US to evoke fear and measured symptoms report and breathing frequency to test the fear response.

Next, we found that acquired fear did not extinguish over the number of trials that were presented. This result is in disagreement

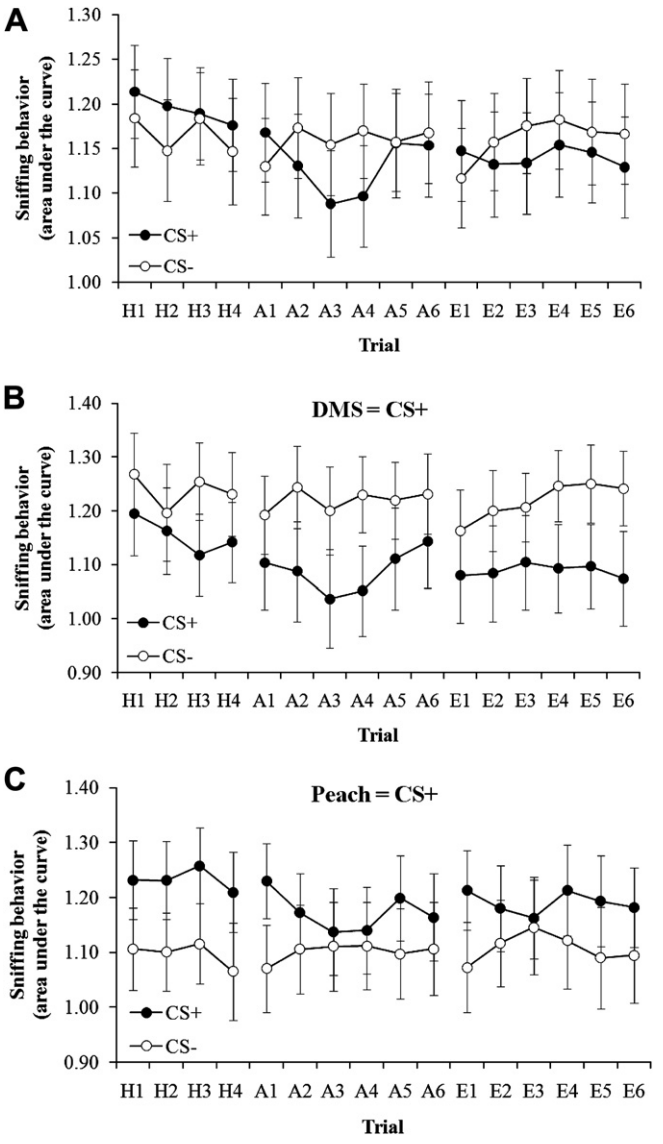

Fig. 5. Change in odor inhalation of CS+ and CS− during habituation (H1–H4), acquisition (A1–A6), and extinction (E1–E6). Data are log transformed means and are shown collapsed across odors (A), and separately for both conditions, with DMS (B) or peach (C) serving as CS+.

with the concept of classical conditioning: if fear for odors is acquired as a result of multiple CS–US pairings, then acquired fear should extinguish after CS-alone presentations (i.e. exposure). These findings then suggest that fear conditioned to odors does not extinguish rapidly.

Furthermore, the results from this study were not in line with the concept of evaluative conditioning: the hedonic value of the originally pleasant CS+ did not significantly decrease as a result of being paired with the US. It should be noted, however, that the trend that can be observed in Fig. 3C suggests that adding more fear acquisition trials might have resulted in a significant decrease in CS+ pleasantness ratings.

Finally, while conditioning of odor to electrical shock led to avoidance of the CS+, as indicated by suppressed inhalation of the CS+ odor on the first four trials, sniffing quickly returned to normal on the last two trials. Together, these results only partially support the classical conditioning account of IEI.

Several observations should be made at this point. As noted, no statistical evidence for extinction was obtained over six CS-alone

trials. Determination of number of trials was based on Blechert, Michael, Vriends, Margraf, and Wilhelm (2007), Effting and Kindt (2007), Milad et al. (2005), and Orr et al. (2000) who showed early extinction of EDR to visual CSs after 6 or less non-reinforced trials. However, for the present study this number of trials turned out to be insufficient.

Furthermore, it was noted that during acquisition EDRs decreased from trial 2 to trial 6. This observation is not uncommon (e.g., Blechert et al., 2007; Milad et al., 2005; Orr et al., 2000). A plausible explanation is that this is due to *habituation*. Habituation is the progressive decline in a defensive response when it is repeatedly reevoked by the same stimulus (Marks & Tobena, 1989). Habituation to the US is not uncommon during classical conditioning and a natural phenomenon that serves to keep the organism alert to novel stimuli. Still, acquisition was successful as is evident from the statistical analyses. Likewise, close inspection of the pattern of responses in Fig. 2C suggests that the intervention of leaving out the US at the first extinction trial had little effect on EDR, as the downward course that had set in during acquisition is showing similar progress during extinction. Thus, the pattern of results may be one of acquisition and habituation rather than acquisition and extinction.

Next, avoidance of the CS+ as indicated by reduced sniffing was only encountered on the first 4 trials, then disappeared as sniffing returned to normal on trials 5 and 6. There are similarities with Fannes et al. (2008) in the sense that an inspection of their Fig. 2 reveals that across three presentations of the CS+ odor minute ventilation in ml/min measured over three 10 s windows first declines and then invariably increases over the last 10 s window. The effect thereupon extinguished. Thus, while avoidance may set in rapidly it is not of a long duration. Whether habituation to the CS+ underlies this effect is at present unclear.

Finally, the DMS odorant was accidentally mixed to be more intense than the peach odorant – the intention was to use iso-intense stimuli – which could have confounded the results. To check whether variance in the results can in fact be attributed to noise due to intensity differences, all analyses were conducted with $\Delta_{\text{INTENSITY}}$ – i.e. the difference in initially perceived intensity between the odorants during the habituation phase – as covariate. The mixing error apparently did not affect our results, as the results of these analyses were no different from those of analyses carried out without the covariate.

In conclusion, we set out to demonstrate that odors can be conditioned to fear. A fear conditioning model with odors as CS may improve our understanding of how IEI develops. We found evidence for classical conditioning only in so far that we demonstrated successful conditioning of both pleasant and unpleasant odors to fear. Our findings regarding fear extinction and behavioral avoidance, however, did not support the model. All in all, the evidence from the present study is insufficient to propose fear conditioning as a model for IEI.

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